Ago2 immunoprecipitation identifies predicted microRNAs in human embryonic stem cells and neural precursors.

Journal: PLoS One

Publication Year: 2009

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PubMed link: 19784364

Funding Grants: CIRM Type I Comprehensive Training Program

Public Summary:
MicroRNAs are required for maintenance of pluripotency as well as differentiation, but since more microRNAs have been computationally predicted in genome than have been found, there are likely to be undiscovered microRNAs expressed early in stem cell differentiation. METHODOLOGY/PRINCIPAL FINDINGS: SOLiD ultra-deep sequencing identified >10(7) unique small RNAs from human embryonic stem cells (hESC) and neural-restricted precursors that were fit to a model of microRNA biogenesis to computationally predict 818 new microRNA genes. These predicted genomic loci are associated with chromatin patterns of modified histones that are predictive of regulated gene expression. 146 of the predicted microRNAs were enriched in Ago2-containing complexes along with 609 known microRNAs, demonstrating association with a functional RISC complex. This Ago2 IP-selected subset was consistently expressed in four independent hESC lines and exhibited complex patterns of regulation over development similar to previously-known microRNAs, including pluripotency-specific expression in both hESC and iPS cells. More than 30% of the Ago2 IP-enriched predicted microRNAs are new members of existing families since they share seed sequences with known microRNAs. CONCLUSIONS/SIGNIFICANCE: Extending the classic definition of microRNAs, this large number of new microRNA genes, the majority of which are less conserved than their canonical counterparts, likely represent evolutionarily recent regulators of early differentiation. The enrichment in Ago2 containing complexes, the presence of chromatin marks indicative of regulated gene expression, and differential expression over development all support the identification of 146 new microRNAs active during early hESC differentiation.

Scientific Abstract:
BACKGROUND: MicroRNAs are required for maintenance of pluripotency as well as differentiation, but since more microRNAs have been computationally predicted in genome than have been found, there are likely to be undiscovered microRNAs expressed early in stem cell differentiation. METHODOLOGY/PRINCIPAL FINDINGS: SOLiD ultra-deep sequencing identified >10(7) unique small RNAs from human embryonic stem cells (hESC) and neural-restricted precursors that were fit to a model of microRNA biogenesis to computationally predict 818 new microRNA genes. These predicted genomic loci are associated with chromatin patterns of modified histones that are predictive of regulated gene expression. 146 of the predicted microRNAs were enriched in Ago2-containing complexes along with 609 known microRNAs, demonstrating association with a functional RISC complex. This Ago2 IP-selected subset was consistently expressed in four independent hESC lines and exhibited complex patterns of regulation over development similar to previously-known microRNAs, including pluripotency-specific expression in both hESC and iPS cells. More than 30% of the Ago2 IP-enriched predicted microRNAs are new members of existing families since they share seed sequences with known microRNAs. CONCLUSIONS/SIGNIFICANCE: Extending the classic definition of microRNAs, this large number of new microRNA genes, the majority of which are less conserved than their canonical counterparts, likely represent evolutionarily recent regulators of early differentiation. The enrichment in Ago2 containing complexes, the presence of chromatin marks indicative of regulated gene expression, and differential expression over development all support the identification of 146 new microRNAs active during early hESC differentiation.